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A STUDY OF THE AGGLUTINATION AND CULTURAL RELATIONSHIP OF MEMBERS OF THE SO-CALLED STREPTOCOCCUS-VIRIDANS GROUP *

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The increasing importance of the nonhemolytic types of streptococci, particularly in focal infections with systemic disease, led us to undertake this study. Such study seemed especially desirable as little information is available as to the immunity relationships of members of this group, most of the investigations having been limited to the determination of biologic characteristics.

The cultures studied included a series known to be pathogenic for man, having been isolated from the blood stream in endocarditis and from other definitely pathologic conditions. For comparison, a series of strains was isolated from the tonsils of healthy individuals. The fundamental criteria used in selecting the cultures were typical streptococcus morphology, especially when they were grown in broth, insolubility in bile or bile salts, and absence of hemolysis as determined by the addition of broth cultures to washed blood cells and the development of greenish color on blood-agar plates. These tests were repeated to eliminate any false negative results. A few strains not in this category were included for comparative purposes in bringing out certain special points.

The strains were then studied to determine their fermentative characteristics, inulin, mannite, raffinose, and salicin being used, and to determine their action on washed blood cells—that is, whether they produce methemoglobin or not.[1] The results with respect to fermentation are given in the table.

The determination of the action on washed blood cells did not yield satisfactory results. When all the cultures were tested at one time, varying grades of methemoglobin-production were present, giving an unbroken series of gradations between the two large groups, of

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* Received for publication August 29, 1916. Part of a study of mouth infections which had its inception in the interest and financial aid of Dr. Arthur H. Merritt.

[1] Lyall: Jour. Med. Research, 1914, 30, p. 487.

sharply positive or definitely negative. The results on different tests were in general alike. Individual false negative results, however, were not infrequent. On the other hand, there was an indication in some instances that an ability to produce methemoglobin which had been dormant, had developed. In some cases a slight discoloration of the supernatant fluid suggested traces of hemolysis as well as methemoglobin-production. With 3 cultures this brownish hemolysis was very distinct, tho one of the strains had had no action on washed cells in a test several months previously, and the other two had been described as green-producing types without hemolysis at the time of isolation.

The production of methemoglobin therefore did not sharply divide the nonhemolytic streptococci into 2 groups. The quantitative character of the results may have been due to the fact that the conditions for the production of methemoglobin were not satisfactory, especially as false negative results were not uncommon. Unless the reaction can be made more uniform and sharper in results, much of its value is lost. At least it cannot be used as the basis for a primary grouping of streptococci.

The agglutination tests were made with broth cultures and immune rabbit sera. In most instances it was not necessary to shake the cultures mechanically if the sediment in the broth was carefully avoided. A varying degree of spontaneous agglutination was encountered. This varied from day to day and in most instances could be overcome by shaking. One culture, Endocarditis 14, which was persistently and strongly self-agglutinating, could not be used even when shaken. The normal content of agglutinins was determined in each rabbit serum used and this value deducted in tabulating the results.

It may be of value to give the method used in producing the agglutinating sera, as we have been able, by this method, to produce serum of high titer with great uniformity. The injections were given intravenously and daily, unless Sunday intervened, according to the following scheme, suspensions heated to 53 C. being employed at first, and then live cultures. The strains were grown on agar slants with a drop of citrated horse blood on the surface. The dosage averaged as follows in terms of total growth on the agar slant: heated, ¼, ½, ¾, ¾; live, ½, ½, 1. The last dose was continued to make 2 weeks of injections. The rabbits were bled from 4 to 5 days after the last injection.

The agglutination results have been combined in one table, the percentage values of the results being given. There seems to be no need

TABLE 1

RESULTS OF THE STUDY OF THE AGGLUTINATION AND CULTURAL RELATIONSHIP OF MEMBERS OF THE STREPTOCOCCUS-VIRIDANS GROUP

Strains and Origin		Inulin	Mannite	Raffinose	Salicin	Blood
Endocarditis	1	—	—	—	+	+
	2	—	—	—	+	+
	3	—	—	—	+	+
	4	—	—	—	+	±
	5	+	—	+	..	+
	6	—	+	+	+	+
	7	+	—	+	+	±
	8	+	+	+	+	—
	9	—	—	+	—	+
	10	+	—	—	+	±
	11	—	—	—	—	+
	12	+	—	+	—	+
	13	+	—	+	+	±
	14	+	—	+	+	$\underline{\pm}$
	15	—	—	—	+	+
Meningitis	1	—	+	—	+	H^1
	2	—	—	—	—	$\underline{\pm}$
Apical Abscess	1	—	—	+	+	+
	2	—	—	—	+	—
	3	—	—	—	+	—
	4	—	—	+	—	+
	5	+	—	+	—	$\underline{\pm}$
M. rheumaticus (Beattie)		—	+	+	+	+
Acute rheumatism		—	+	—	+	H^1
Rheumatism and pericarditis		+	—	—	+	±
Arthritis (foot)		—	+	+	+	+
Prostate (arthritis)		—	+	+	+	±
Furuncle (neck)		—	—	+	+	—
Chronic bronchitis		—	—	+	+	H^2
Tonsils, normal individuals (the initial indicates the individual; the number, the strain)	V_3	—	—	+	—	—
	K_1	+	—	+	+	+
	K_2	—	—	+	+	—
	N_1	—	—	+	+	—
	N_2	—	—	+	—	—
	T_1	—	—	—	—	+
	W_1	+	—	+	+	+
	W_2	+	—	+	+	—
	W_3	+	—	+	+	—
	W_4	+	—	+	+	—
	W_5	+	—	+	+	—
	H_1	+	—	—	—	+
	H_4	+	—	—	—	+
	H_5	—	—	+	+	—
	B_2	+	—	+	+	+
	B_3	—	—	—	—	+
	B_4	—	—	+	+	+
	M_2	—	—	+	—	+
	M_5	+	—	+	+	—
	S_3	+	—	+	+	—
	S_4	—	—	+	+	—
	S_5	—	—	—	—	+
	Ta_2	—	—	+	+	+
	Ta_3	+	—	—	—	+
	Ta_4	+	—	—	—	+
Titer of sera		..	..	..	..	..

EXPLANATION OF TABLE

Blood.—In this column + means definite methemoglobin-production; ±, moderate methemoglobin-production; $\underline{\pm}$, slight or doubtful methemoglobin-production; —, no change; H, hemolysis (1) reddish brown, (2) deep garnet.

Agglutination.—To economize space and to make the results directly comparable and easy of interpretation, percentage values are given. The actual results were first reduced to numeric values according to the dilution results. Thus a complete reaction at 1:200 was given a value of 200. A half complete reaction was given a numeric value of half the dilution, and so on. The latter procedure was followed only in low dilutions, exceptionally in higher dilutions where a partial reaction persisted. In these instances the numeric

TABLE 1—*Continued*

RESULTS OF THE STUDY OF THE AGGLUTINATION AND CULTURAL RELATIONSHIP OF MEMBERS OF THE STREPTOCOCCUS-VIRIDANS GROUP

Agglutinating Sera									
Endocarditis No. 1	Endocarditis No. 2	Endocarditis No. 6	Endocarditis No. 10	Endocarditis No. 11	Endocarditis No. 14	Apical Abscess No. 2	Tonsil W_2	Tonsil K_2	Tonsil M_5
100	100	0	0	0	—	0	0	0	0
50	100	0	0	0	—	0	0	0	0
55	100	0	0	0	—	0	0	0	0
0	0	0	0	0	+	0	0	0	0
0	0	0	1	0	+	0	0	0	0
0	0	100	0	0	—	0	0	0	0
1	0	1	0	0	—	0	2	2*	10
0	0	0	0	0	—	0	0§	0*	0†
1	0	0	1	0	+	0	33	10	33
1	0	0	100	0	—	0	0	0	0
1	0	0	12	100	+	0	10*	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
S	S	S	S	S	S	S	S	S	S
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	100	0	0	0
0	0*	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
3	5	0	2	0	—	0	12	5	33
0	0	0	1	0	—	0	0	0	0
6	0	0	0	1	+	0	0*	0*	0†
6	20	1	1	0	—	0	5	0	7
1	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
1	0	0	0	0	+	0	50§	66*	33‡
25	50*	0	2	1	+	0	150	100	100
20	32	0	0	0	—	0	20	7	66
1	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
1	5	0	0	0	+	0	25*	1*	0
20	50*	0	1	1	+	0	100	8	10
10	32	0	3	0	+	0	100	33	66
0	0	0	3	0	+	0	12*	2*	10†
5	20	0	3	0	+	0	12	10*	10
0	0	0	0	1	—	0	0	0	0
1	0	0	0	0	—	0	0	0	0
1	1	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	12	2	10
1	1	0	0	0	—	0	12	3*	17
20	10	0	0	0	—	0	12	3	17
0	0	0	1	1	+	0	0*	0*	2*
1	1	1	1	0	+	0	12	1*	100
10	5	0	0	0	+	0	2†	2	10
1	0	0	0	0	+	0	0†	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
0	0	0	0	0	—	0	0	0	0
400	200	800	600	400	?	180	400	600	600

values may be too high, but for our purpose this is preferable to underestimation. The figures so obtained were then converted into percentage values using the figure 100 for the homologous strains. The highest dilution (titer of sera) giving a complete reaction with the homologous strain, is given. In the case of the serum immunized to Endocarditis 14, the titer not being determinable (S = spontaneous agglutination), the presence or absence of agglutination is noted only with the symbols + and —. Action of normal agglutinins in dilutions over 1:10 are noted as follows: (*) = 1:50, (†) = 1:100, (§) = 1:200, referring to the highest dilution showing any agglutination. In one instance (‡) traces were present up to 1:600.

of giving all the reactions at the different dilutions of each serum separately. In the case of one serum the titer could not be determined because of spontaneous agglutination, but its action on the other strains is given because the results offer further evidence for deductions made in discussing results.

The agglutination reactions offered at least no basis for grouping of the strains of pathologic origin, tho cross agglutinations were present. The only possible exceptions were the endocarditis strains, Nos. 1, 2, and 3. These, which came to us in this order from Mt. Sinai Hospital, unfortunately were accompanied by no data concerning their origin. However, we feel that they were probably 3 successive isolations from the same case, repeated blood cultures being a common practice at this hospital.

A study of the cross agglutinations yields some interesting data. Serum immunized to Endocarditis Strain 10, as well as sera immunized to other strains, agglutinated Endocarditis Strain 11 to a slight degree, yet serum immunized to Endocarditis Strain 11 did not agglutinate Endocarditis Strain 10, nor the other apparently related strains. The most striking point is that certain strains were agglutinated to a varying degree by many sera, and this was more noticeable among the normal throat cultures. Furthermore, sera immunized to these strains from normal tonsils agglutinated, as a rule, a higher proportion of the tonsillar strains and commonly at much higher dilutions. The sera immunized to the tonsillar strains which were agglutinated by sera immunized to the types of pathologic origin, had no action with few exceptions (Endocarditis Strains 7, 9, and 11, and rheumatism, pericarditis, and furuncle strains) on the latter types. Thus serum immunized to Strain K 2, which was acted upon by most sera, did not agglutinate any of the strains of pathologic origin used in the preparation of those sera. In contrast to this, the three tonsillar strains, K 2, W 2, and M 5, showed a sharp degree of interagglutination. This would suggest a tendency to grouping, if one could explain the fact that they were acted upon by other sera when a reverse action was absent.

On the whole, the data seem to us to justify the conclusion that the types pathogenic for man constitute a heterogeneous group. At least from the practical standpoint, this conclusion seems justified; that is, the possibility of a multiplicity of groups is not excluded by the amount of work we have done. A few of the types from pathologic conditions, but many of the tonsillar types, tended to possess a common agglutinin-binding content but did not necessarily show a common agglutinogenic content, as they did not cause the production of agglutinins active against other strains unless these strains had given evidence, at least to some extent, of possessing a common agglutinin-binding content for several sera. This deduction seems warranted, tho certain factors must be considered in relation to the cross agglutinations. The tonsillar strains showed a greater tendency, numerically, to spontaneous agglutination and a somewhat higher proportion were acted upon by the normal agglutinins present in the rabbit sera. Many strains showed a partial or slight agglutination in a dilution of 1 : 10. A few strains were agglutinated in dilutions over 1 : 10, but only partially. These reactions are noted in the table. The majority of these high reactions occurred with the three sera from rabbits injected with the tonsillar strains. In other words the sera of these rabbits had a higher content of normal agglutinins.

The greater tendency, therefore, of the tonsillar strains to be agglutinated spontaneously, and the greater number of these strains agglutinated in the normal serum, probably contributed in producing the results. The instability of the suspension as evidenced by the tendency to spontaneous agglutination would reveal traces of agglutinins otherwise inactive. The fact that the three rabbits used for the tonsillar strains had a higher normal content of agglutinins is probably in part responsible for the great number of cross agglutinations; that is, having a high content of normal agglutinins, they may have responded quantitatively higher in producing group or common agglutinins. This would again be further intensified when they were acting on the unstable suspensions. Altho these factors probably explain the high figures, they do not invalidate the deduction already given.

The agglutination results are in accord with the enormous multiplicity of types which can be differentiated by the determination of fermentative characteristics. On the other hand, there is no correlation between interagglutinations and fermentative reactions, even where the former are very marked.

There is little in the voluminous literature on streptococcus differentiation that is available for comparison with our results. Floyd and Wolbach[2] determined the interagglutination only of cultures representative of cultural subgroups of the streptococcus group as a whole.

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[2] Jour. Med. Research, 1914, 24, p. 493.

Kligler[3] also employed agglutination in the attempt to correlate the results of this reaction with fermentative characteristics, studying the streptococcus group as a whole. He used sera prepared against 4 representative strains. Serum immunized to one endocarditis strain agglutinated only its homologous strain. A serum immunized to a strain from a normal tonsil agglutinated to some extent, strains from endocarditis, 2 from pleurisy, 1 from a lymph node in scarlet fever, and 1 from an antrum slightly. The only striking cross agglutination was with a strain from acute tonsillitis. We feel that the lack of ability of this last strain to cause hemolysis places it in all probability among the nonpathogenic mouth types. If this is so, he has demonstrated a sharp cross reaction similar to our own. The degree of reverse agglutination was not determined in any case.

SUMMARY

Streptococci of the "viridans" type isolated from pathologic conditions apparently constitute a heterogeneous group. Saprophytic types may show some relationship one to the other, but practically none to the pathogenic type. Therapy, serum or vaccine, to elicit specific results is therefore possible only with autogenous strains.

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[3] Jour. Infect. Dis., 1915, 16, p. 327.